at least one but no more than six nucleotides deleted on the side distal from the spacer sequence.

Claim 10. A DNA construct comprising:

(1) a selective marker gene,

(2) a galactose-inducible growth inhibition sequence,

(3) a pair of FRT (Flp recombinase recognition target) sequences in the same orientation flanking (1) and (2), and

(4) a DNA fragment capable of recombining with a yeast chromosomal DNA located at each end of (3),

wherein said FRT sequences contain the following sequence:

5'-GAAGTTCCTATAC TTTCTAGA GAATAGGAACTTC-3' (SEQ ID NO: 1)

inverted repeat (1) spacer sequence inverted repeat (2)

provided that in each member of said pair of FRT sequences, the inverted repeat distal from the flanked selective marker gene and growth inhibition sequence has at least one but no more than six nucleotides deleted on the side distal from the spacer sequence.

REMARKS

Reconsideration and allowance of the present application are respectfully requested.

In response to the request for sequence compliance wherein those sequences stated at page 24, lines 18 and 19 do not have sequence identification

numbers, the specification has been amended to indicate that the two sequences are SEQ ID NO: 29 and SEQ ID NO: 30. A copy of the Notice to Comply is attached to this Amendment. Please note that the sequence at page 24, line 19 was in error and has been replaced. The replacement sequence at page 24, line 19 is a combination of FRT103-a (SEQ ID NO: 21) and FRT3-a (SEQ ID NO: 19) as supported at page 24, line 15 and 20. No new matter has been added.

Thus, in accordance with 37 CFR 1.821(c), a substitute "Sequence Listing" is submitted herewith, and it is respectfully requested that the Listing be entered into the application in replacement of the sequence listing filed September 26, 2002. The substitute Sequence Listing includes SEQ ID NO: 29 and SEQ ID NO: 30. The Sequence Listing does not include new matter. A copy of the "Sequence Listing" in computer readable form is also submitted herewith, in accordance with 37 CFR 1.821(e), and includes no new matter.

Furthermore, in accordance with 37 CFR 1.821(f), it is submitted that the contents of the paper copy and the computer readable copy of the Sequence Listing are the same.

In response to the rejection to the Abstract at page 3 of the Office Action, the applicants submit herewith a new, single page Abstract which is of proper length.

In response to the Examiner's request for a new Title of this application, the applicants have provided a new, properly descriptive, title as shown above.

Entry of the revised Abstract and Title is respectfully requested.

Regarding the objection to claim 1, and the request for the definition of "FRT" in claim 1, claim 1 has been accordingly amended as shown above. The definition of the term "FRT" is "Flp recombinase recognition target". For

reference, the applicants enclose herewith a copy of Storici et al. (Yeast (1999), 15, 271-283), which was published before the priority date of the present application and is referenced at pages 3-4 of the present specification. The definition of "FRT" is described in line 1 of the abstract of the Storici et al. publication (indicated by an arrow).

Claims 1-8 stand rejected under 35 USC 112, first paragraph, for the reasons stated at pages 3-4 of the Office Action.

The applicants submit that the invention of claim 1 does satisfy the Written Description requirement of Section 112, first paragraph because those of ordinary skill in the art could indeed have carried out the DNA construct having a sequence "substantially identical to" the native FRT sequence.

The applicants point out that Example 1 of the present specification describes that a recombination event actually occurred when yeast was transformed by each DNA construct having deletion mutations, which lacks "at least one but more than six nucleotides" on the side distal from the spacer sequence. Therefore, the applicants are certain that those ordinarily skilled in the art could have practiced the mutations obtained by deletion of nucleotides.

Additionally, please refer to the enclosed copy of the aforementioned Storici et al. (Yeast (1999), 15, 271-283). This is an appropriate publication to show the state of the art as of the priority date since it was published before the priority date of the present application and it is recited in the present specification (pages 3-4). Storici et al. describes the three examples of substitution mutations within the spacer sequence (mutations X, G and H) (see Figure 3 and its explanation in the first paragraph of RESULTS section in page 277 of Storici) and

the recombination event which was actually occurred by using these mutations (see Table 3 and the paragraph bridging pages 277 and 288).

Those of ordinary skill in the art could have carried out the invention relating "substantially identical sequences" by referring to the disclosure of the present specification and the state of the art such as Storici et al. Therefore, the applicants submit that the Written Description requirement for claim 1 is satisfied.

As the examiner noted in the office action, the term "substantially identical sequence" is defined in the present specification as "a sequence that can be recognized by the Flp protein to induce recombination between FRT sequences, such as a nucleotide sequence obtained by modifying the sequence defined above by substitution, deletion or addition of one or several nucleotides" (lines 20-25, page 10). New Claims 9 and 10 are supported by this description.

The applicants respectfully submit that all presently considered claims are fully allowable under Section 112, first paragraph.

Claims 3-8 stand rejected under 35 USC 112, first paragraph, for the reasons stated at pages 5-6 of the Office Action.

With respect to claim 3, the applicants point out that the term "a DNA fragment" in line 5 of claim 3 is the same DNA fragment recited in claim and accordingly, the applicants have amended claim 3 as proposed by the Examiner in paragraphs 3 of Section 9, page 5 of the Office Action.

Claim 3 has been further amended in response to the Examiner's comments on the terms "a selective marker", i.e., at line 9 of claim 3 and "a pair of FRT sequences" at line 12 of claim 3.

Regarding claim 4, this claims has been amended as shown above, as suggested by the Examiner.

With respect to claim 7, this claim has been amended to recite method steps and thus the applicants submit that the amended Claim 7 is clear and definite.

The applicants respectfully submit that all presently considered claims are fully allowable under Section 112, second paragraph.

The applicants respectfully traverse the rejection of claim 8 under 35 USC 102(b) in view of Omura et al. This reference does not anticipate the presently claimed invention or make it obvious.

The problems to be solved by the invention of Omura is "to decrease the presence of hydrogen sulfide in a beer brewing process by adapting a gene (such as MET25) coding for yeast O-acetylhomoserine sulfhydrylase so that the gene is expressed, preferably constitutively, in a brewing yeast". Omura solves the problems as described above by a process for production of beer using "yeast belonging to the genus Saccharomyces transformed with a recombinant vector comprising a promoter from a gene constitutively expressed in yeast, located upstream of a structural gene coding for yeast O-acetyphomoserin sulfhydrylase, and capable of a constitutive expression of a large amount of O-acetyphomoserin sulfhydrylaseresulting in a decrease of hydrogen sulfide generated in a beer brewing process".

In contrast, the invention of claim 8 is directed to a beer using a transformed yeast lacking a selective marker gene. <u>The invention is not directed to a beer in which generation of hydrogen sulfide in a beer is decreased.</u>

As the applicants described in page 1 of the present specification, the problems to be solved by the present invention is "to remove and reuse selective marker genes to repeatedly transform the same strain because few classes of

drug resistance genes can be efficiently used in yeast" . The present invention solves the problems as described above by providing the DNA construct as claimed in claim 1 "for preparing a transformant lacking a selective marker gene and efficiently expressing a desired gene of interest". The invention of claim 8 employs the DNA construct of claim 1 to produce a beer.

There is no description nor suggestion in Omura about the problems to be solved by the present invention, or the DNA construct as claimed in claim 1 for solving the problems. The invention of claim 8 which is a beer resulting from the use of yeast transformed with the DNA construct of claim 1 is not suggested by Omura and is certainly not identical to the invention of beer as described in Omura.

Accordingly, the presently claimed invention is no where disclosed, suggested or made obvious by the teachings of Omura. The presently claimed invention is not only allowable under Section 102(b) in view of Omura, but is also allowable under Section 103(a) in view of Omura.

The applicants respectfully traverse the rejection of claims 1-6 under 35 USC 103(a) over Ashikari et al. in view of Kawahata et al. None of the cited references make the presently claimed invention to be obvious.

The present invention employs the use of FRT sequences/Flp system for the recombination of yeast and for the excision of selective marker genes.

Ashikari describes an invention using the Zygosaccharomyces rouxii-derived site-specific recombination system R/RS in Saccharomyces cerevisiae. The reference describes problems in the invention of Ashikari that, since the introduction of a foreign gene for recombinase (R gene) is involved in the

invention, the excision efficiency of the selective marker gene varies with the strain of yeast to be transformed.

Kawahata describes excision of selective marker genes using growth inhibition sequence (GIN sequence). The method of Kawahata successfully removes the selective marker gene at an efficiency of 96% or more. However, there are problems in the invention of Kawahata wherein this method is not suitable for commercial use because the selective marker gene is excised by homologous recombination to leave an unnecessary long sequence as an excision mark of the selective gene on the chromosome.

There is no disclosure or suggestion in Ashikari or Kawahata for using the DNA construct as claimed in claim 1 and the FRT/Flp system of the present invention to recombine yeast. Neither cited reference describes or suggests or provides the motivation to realize the concept of FRT/Flp system. Even if those ordinary skill in the art combined the disclosure of these citations, they could not have resulted in the invention of present claims 1-6.

Furthermore, the present specification describes that, by using the invention, it is possible to remove the selective marker gene at high efficiency and to shorten the unnecessary sequence as an excision mark of the selective gene on the chromosome. Specifically, the presently claimed invention successfully removes the selective marker gene at an efficiency of almost 100% when the DNA construct includes FRT3-FRT103 sequence as FRT sequence and GIN sequence (see Example 3), although the selective marker is excised at a few % in yeast recombined with recombination system R/RS. The unnecessary sequence as an excision mark of the selective gene on the chromosome can be about 20-40 bp in yeast recombined with FRT/Flp system according to the present invention, while

the unnecessary sequence on the chromosome is about 1.2 kbp in yeast according to the invention of Kawahata.

Thus, these are significant results which can not be expected from the references of Ashikari or Kawahata, or the combination of these references.

Moreover, the applicants submit that those of ordinary skill in the art would not have combined these references since there are differences in the problems to be solved and the means for solving the problems. Thus, the applicants assert that the present combination of references is not tenable and should be withdrawn.

Even if those of ordinary skill in the art combined the disclosure of these citations, they could not have resulted in the invention of claims 1-6. Further, the present invention demonstrates the significant effects which can not be expected from the inventions of the citations. Therefore, the presently claimed invention is fully allowable under Section 103(a) in view of Ahikari taken with Kawahata.

The applicants respectfully traverse the rejection of claims 1-8 under 35 USC 103(a) over Ashikari et al. in view of Kawahata et al. and further in view of Omura. None of the cited references make the presently claimed invention to be obvious.

The teachings of Ashikari and Kawahata have been discussed above and thoroughly distinguished from the presently claimed invention. The applicants submit that the teachings of Omura do not remedy the defects of Ashikari and Kawahata.

As discussed above, there is no suggestion nor description in Ashikari or Kawahata for using the DNA construct as claimed in claim 1 and the FRT/Flp system of the present invention to recombine yeast. Neither references describe

or suggest or provide motivation to result in the presently claimed invention involving FRT/Flp system.

As also described above, Omura discloses a concept irrelevant to the present invention, considering the differences in the problems to be solved and the means for solving the problems. The applicants submit that Omura is not appropriate to be cited for rejecting the present invention.

Thus, those of ordinary skill in the art could not have combined these three citations since there are differences in the problems to be solved and the means for solving the problems. The applicants submit that those of ordinary skill in the art would not have combined these references since there are differences in the problems to be solved and the means for solving the problems. Thus, the applicants assert that the present combination of references is not tenable and should be withdrawn.

Even if those of ordinarily skill in the art combined the disclosure of these citations, they could not have resulted in the invention of claims 1-8. Further, the present invention demonstrates the significant effects which can not be expected from the inventions of the citations.

Accordingly, the applicants submit that the presently claimed invention is fully allowable under Section 103(a) in view of Ashikari, Kawahata and Omura taken alone or in combination.

The applicants respectfully traverse the rejection of claims 1-6 under the judicially created doctrine of obviousness-type double patenting over claims 1-7 of USP 5,965,444, in view of Kawahata et al.

As thoroughly discussed above, Ashikari et al. (corresponding to U.S. Patent No. 5,965,444) claims a DNA construct using the Zygosaccharomyces

rouxii-derived site-specific recombination system R/RS (claim 1) and the method for transforming yeast using the DNA construct of claim 1 (claim 4). Specifically, claim 1 of USP '444 describes that "A DNA construct comprising an R gene positioned under the control of an inducible promoter, and an expressible selective maker wherein the R gene and the selective marker are flanked by a pair of R sensitive sequences oriented in the same direction so as to form a removing unit...".

In contrast, the presently claimed invention recites a DNA construct using FRT/Flp recombination system and GIN sequence (claim 1) and the method for transforming yeast using the DNA construct of claim 1 (claim 3).

Thus, since the claimed invention of the present application is not suggested or made obvious by the claims of USP '444, the claimed invention of the present application is fully allowable under the Double Patenting rejection based on the claims of USP '444. Withdrawal of this rejection is accordingly requested.

In view of the above and the attached sequence listing, Abstract, and technical reference, it is believed that this application is in condition for allowance and a Notice to that effect is respectfully requested.

Respectfully submitted,

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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

Please amend the title as follows:

-- [METHOD OF BREEDING] DNA CONSTRUCT FOR TRANSFORMING A YEAST--

IN THE SPECIFICATION:

Please amend the present specification as follows:

Paragraph at page 24, starting at line 17

[3b 103b

3W 5' -AATTCTCCTATTCTCTAGAAAGTATAGGAA- 3'
3' -GAGGATAAGAGATCTTTCATATCCTTTCGA- 5'

3a 103a]

3b 103b

3W 5' -AATTCTCCTATTCTCTAGAAAGTATAGGAA- 3' (SEQ ID NO: 29)
3' -AGCTTTCCTATACTTTCTAGAGAATAGGAG- 5' (SEQ ID NO: 30)

103a 3a

IN THE CLAIMS:

Proposed Amendments To Claims 1, 3, 4 and 7 Showing Deletions And Insertions.

Claim 1. (Amended) A DNA construct comprising:

(1) a selective marker gene,

(2) a galactose-inducible growth inhibition sequence,

(3) a pair of FRT (Flp recombinase recognition target) sequences in the same orientation flanking (1) and (2), and

(4) a DNA fragment capable of recombining with a yeast chromosomal DNA located at each end of (3),

wherein said FRT sequences contain the following sequence:

5'-GAAGTTCCTATAC TTTCTAGA GAATAGGAACTTC-3' (SEQ ID NO: 1)

inverted spacer inverted

repeat (1) sequence repeat ([1] 2)

or a sequence substantially identical to said sequence,

provided that in each member of said pair of FRT sequences, the inverted repeat distal from the flanked selective marker gene and growth inhibition sequence has at least one but no more than six nucleotides deleted on the side distal from the spacer sequence.

3. A method for transforming a yeast of the genus Saccharomyces, comprising:

(1) transferring the DNA construct of claim 1 into yeast cells to integrate said DNA construct into a yeast chromosome by recombination between [a] the two DNA [fragment] fragments [capable of recombining with a yeast chromosomal DNA present in said DNA construct] and the yeast chromosomal DNA,

(2) selecting yeast cells transfected with said DNA construct based on the expression of [a] the selective marker gene contained in said DNA construct,

(3) culturing said cells in a non-selective medium to induce recombination between [a] the pair of FRT sequences contained in said DNA construct, thereby excising the selective marker gene, and

(4) culturing said cells in a medium containing galactose to select growable yeast cells.

Claim 4. (Amended) The method of claim 3 [comprising inserting a gene of interest into a yeast chromosome using a DNA construct containing said gene of interest inserted between a DNA fragment capable of combing with a yeast chromosomal DNA and] wherein said DNA construct further comprises a gene of interest between said DNA fragment and said FRT sequence adjacent to said fragment.

Claim 7. (Amended) A method for producing a beer comprising [using] the following steps:

adding the yeast of the genus Saccharomyces of claim 6 to wort, and fermenting said wort containing the yeast.